Contribution of Stem Cells to Kidney Repair

Benedetta Bussolati, Peter Viktor Hauser, Raquel Carvalhosa and Giovanni Camussi*

Renal and Vascular Physiopathology Laboratory, Department of Internal Medicine, Molecular Biotechnology Centre and Research Centre for Molecular Medicine, University of Torino, Torino, Italy

Abstract: A current explanation for development of chronic renal injury is the imbalance between injurious mechanism and regenerative repair. The possibility that stem cells contribute to the repair of glomerular and tubular damage is of great interest for basic and translational research. Endogenous bone marrow-derived stem cells have been implicated in the repair of renal tissue, although the lineage of stem cells recruited has not been determined. If endogenous bone marrow-derived stem cells repopulate injured nephrons directly or act indirectly over a paracrine/endocrine mechanism remains also controversial. Therapeutic administration of exogenous bone marrow derived stem cells in animal models of acute renal injury suggests that a stem cell-based therapy may improve the recovery of both glomerular and tubular compartments. Whereas the therapeutic benefit of sorted hematopoietic stem cells remains uncertain, several studies showed a beneficial effect of mesenchymal stem cell administration in models of acute tubular injury and of endothelial progenitors in acute glomerular injury. Recent studies demonstrate the presence of resident stem cells within the adult kidney. These cells are capable, when injected in animals with acute tubular injury, to localize to renal compartments and contribute to regeneration. This review summarizes the current literature on the physiological role of endogenous stem cells in renal regeneration and on the therapeutic potential of exogenous stem cell administration. Moreover, critical points that still need clarification, such as the homing mechanisms of stem cells to injured tissue, the secreted factors underlying the paracrine/endocrine mechanisms and the long-term behaviour of in vivo administered stem cells, are discussed.

Keywords: Models of renal disease, renal injury, renal repair, mesenchymal stem cells, cell therapy, renal regeneration.

INTRODUCTION

The kidney is a highly complex organ, composed of more than 30 different cell types in different compartments, such as tubular epithelial cells, interstitial cells, glomerular cells and cells of the vasculature. These cells and tissues differ in their proliferation rate, turnover and regenerative potential. Even the different compartments of the nephron, the smallest functional unit of the kidney, exhibit different regenerative capacity. Glomerular visceral epithelial cells, or podocytes, are terminally differentiated and their proliferation rate is virtually zero [1]. If podocytes are lost due to necrosis, apoptosis or detachment, they are not replaced by proliferation of a neighboring podocyte [2]. Proximal tubular cells on the other hand, have a slow cell turnover under normal physiological conditions. After a damaging event, the tubular cells respond with diffuse proliferation. Recent studies showed the possibility that stem cells (SC) either derived from the bone marrow (BM) or resident in the renal tissue itself, may contribute to renal recovery after damage. The aim of the present review is to illustrate the current evidence about the contribution of endogenous SC in the regeneration of the tubular and glomerular compartments after acute kidney injury (AKI) or glomerular damage, respectively. In addition, recent results of the therapeutic administration of exogenous SC in experimental models of acute tubular and glomerular injury will be discussed.

ACUTE KIDNEY INJURY

The term acute kidney injury (AKI) describes a sudden and prolonged reduction of the renal glomerular filtration rate causing the retention of metabolites. Characteristics of the injury are tubular necrosis and apoptosis [3], changes of the filtration barrier, glomerular misfiltration, vasoconstriction and tubular obstruction, as well as interstitial swelling and activation of proteolytic enzymes [4]. The world wide incidence rate of AKI is unclear because of disparities in diagnostics, classification and regional variation [5], but a great number of patients with AKI require hemodialysis [6]. Up to 30% of all patients admitted to intensive care units are affected by primary or secondary AKI [7]. The mortality rate of patients requiring dialysis as a result of AKI is twice as high compared to patients without AKI [7, 8]. Clinical management of AKI has improved significantly over the last years, but a specific therapy to improve renal function after AKI has not been developed yet. Complications arise from the inability of the kidney to regenerate lesions with functional tubular epithelial cells [9]. Failure to replace damaged cells with epithelial cells gives rise to tubulo-interstitial fibrosis and scarring, increasing the susceptibility for chronic renal injury.

Bone Marrow Derived Stem Cells (BMSC) in the Regeneration of AKI

Differentiation of BMSC into cells of non-hematopoietic origin has been described in several in vivo studies and gave rise to the thought that the BMSC population could be involved in tissue turnover and regeneration [10-14]. The possibility that BMSC might functionally contribute to renal tubular regeneration is still matter of debate. In order to evaluate a possible mobilization of BMSC to the

*Address correspondence to this author at the Cattedra di Nefrologia, Dipartimento di Medicina Interna, Ospedale Maggiore S. Giovanni Battista, Corso Dogliotti 14, 10126 Torino, Italy; Tel: +39 011 6336708; Fax: +39 011 6631184; E-mail: giovanni.camussi@unito.it

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kidney. Poulsom and colleagues [15] studied the renal engraftment of BM-derived cells in female mice that received male BM transplants. By in situ hybridization, they were able to detect Y-chromosomes carrying cells in tubular epithelial cells and podocytes. Similar studies have confirmed the capacity of BM-derived cells to differentiate into renal epithelial cells [16] and mesangial cells [17, 18]. Integration and differentiation of BMSC into renal cell types was also observed after AKI [19-21]. However, the low percentage of integration seen [21, 22] raised doubt on the true importance of BMSC in tubular repair. In fact studies using transgenic mice expressing enhanced GFP and the bacterial LacZ gene, have shown that only 8.3 ± 3.2% of the newly integrated renal cells was derived from the BM [21], or that BM-derived cells were absent at all [22]. On the contrary, most of the proliferating tubular epithelial cells were host-derived [21]. These results support the idea that tubular regeneration is achieved through dedifferentiation and proliferation of surviving renal epithelial cells. A more recent work by Humphreys et al. [23] supports this theory. Using a genetic tag, they labelled mesenchyme-derived renal epithelial cells (i.e. from the Bowman's capsule to the junction of the connecting segment and collecting duct) whereas the entire interstitial compartment remained unlabeled [23]. In a model of ischemia-reperfusion they found that roughly 95% of the regenerated tubular epithelial cells carried the genetic tag and no dilution of the cell-fate marker was observed, indicating that tubular repair was mainly a result of tubular proliferation. A small percentage of extrarenal contribution or cell to cell fusion can not be excluded.

There are studies involving cell fusion as a mechanism of BM-derived cell epithelial differentiation [24]. Several authors addressed the possibility that BM-derived cells fuse with tubular epithelial cells and showed that individual cells or small patches of tubule epithelium can be derived by cell:cell fusion. In a model of folic acid-induced AKI, Fang et al. [19] showed that among BM-derived cells integrated into the renal epithelium (around 10% of the cells) some of them showed signs of fusion to the renal tubular epithelium. In an animal model of fumarylacetoacetate hydrolyase (Fah) deficiency, Fah+ transplanted BM cells significantly replaced damaged proximal tubular epithelium. In this experiment, up to 50% BM-derived epithelial cells were generated by cell fusion and not by transdifferentiation [25]. Recently, Li et al. [26] in a model of ischemia-reperfusion injury found that BM-derived cells were fused with renal epithelial cells, but the low frequency of this event did not account for the majority of the BM-derived cells within the renal epithelium. The authors transplanted male BM from a mouse carrying a loxP flanked reporter gene into a female mouse that expressed Cre-recombinase. Using the expression of the reporter gene as a marker, they found only 0.066% fused cells of the 1.8% of tubular cells derived from BM. In contrast, using detection by chromosome analysis they found 3.8% of the BM-derived cells. However, the relevance of this phenomenon deserves further investigation.

Despite different results regarding integration of BMSC, these studies confirmed an effective improvement of renal function provided by the BM-derived SC, by comparing irradiated- and non-irradiated animals or irradiated animals with reconstituted BM [23]. The identity of the endogenous BM-derived SC that home to injured kidney and the differential involvement of hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are still unclear, but it may be relevant for designing therapies based on BM-derived SC mobilization. This point has been recently addressed by a study of Fang and collaborators in a model of HgCl₂-induced acute tubular injury [27]. By using differential detection of BM transplanted cells (GFP for HSC and Y chromosome for MSC), they found that HSC and not MSC engraf in the renal tubules and that these cells were able to proliferate after AKI. In addition, few MSC were detected in the renal interstitium, but their role was not determined.

The results on BMSC mobilization in experimental models are discordant. Toegel et al. [28] reported that HSC mobilization has a potential detrimental effect on kidney regeneration and is further associated with increased severity of renal failure and mortality after ischemia-reperfusion injury. This may be explained by the induction of a marked granulocytosis. In contrast, Fang et al. [19] and Iwasaki et al. [29], using folic acid and cisplatin induced AKI, demonstrated the renoprotective effect of BMSC mobilization with G-CSF or the combination of G-CSF and M-CSF. This might be the effect of the absence of granulocytosis in these models. Further a myelosuppressive effect of cisplatin itself cannot be ruled out. Recently, erythropoietin was shown to mobilize a stromal population from the BM with renoprotective effect in acute renal damage [30].

**Administration of MSC and the Recovery from AKI**

Several studies have demonstrated that the administration of *in vitro* expanded SC may protect and reverse AKI in different models [31-35]. Necrotic lesions in the proximal and distal tubules may support migration of MSC to the kidney. Detection of the injected MSC in the kidney, revealed a low number of MSC within the regenerated tubule despite a high number of proliferating cell nuclear antigen-positive tubular cells, suggesting a stimulatory effect of MSC on surviving resident tubular cells [33]. Indeed, injected MSC were mostly found in the interstitium rather then in the tubules or endothelium [35].

Yokoo et al. demonstrated that GDNF-expressing MSC cells, injected into the intermediate mesoderm at the nephrogenic site of the embryo, differentiate into kidney structures [36]. In contrast, MSC injected into the developing metanephros did not integrate and differentiate, which suggests that exposure of the MSC to the repertoire of nephrogenic signals is required to start development of the metanephros. Actual homing of MSC to the lesions might depend on the severity of injury. Integrated cells could be overlooked after their differentiation to endothelial cells, as Broekema et al. [37] demonstrated on BM-derived cells in an ischemia-reperfusion injury model. Duffield et al. [22] also studied ischemia-reperfusion induced AKI and found that injected MSC had a beneficial effect only when the cells were kept under conditions favouring differentiation into endothelial cells. They also could not detect MSC integrated into tubules. Despite problems in comparing the severity of damage and different injury models, there is strong evidence that transplantation of MSC after AKI has a beneficial effect on the regeneration ability of the kidney (Table I). Currently
Table 1. Therapeutic Administration of Stem Cells in Experimental Models of Acute and Chronic Renal Damage

<table>
<thead>
<tr>
<th>Model</th>
<th>Therapeutic Effects</th>
<th>Side/Negative Effects</th>
<th>Refs.</th>
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<tbody>
<tr>
<td>Acute Tubular Injury</td>
<td>Morphological/functional recovery</td>
<td>Adipocyte differentiation</td>
<td>[31, 32]</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
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<tr>
<td>Cysplatin</td>
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<tr>
<td>Ischemia-riperfusion</td>
<td></td>
<td></td>
<td>[22, 33, 35, 37, 38]</td>
</tr>
<tr>
<td>Acute Glomerular Injury</td>
<td>Recovery and vascularization</td>
<td>Progression into glomerulosclerosis</td>
<td>[64]</td>
</tr>
<tr>
<td>Thy-1 glomerulonephritis</td>
<td></td>
<td></td>
<td>[60, 61]</td>
</tr>
<tr>
<td>Chronic kidney Injury</td>
<td>Recovery</td>
<td>No improvement of renal function</td>
<td>[63]</td>
</tr>
<tr>
<td>Anti-mesangial Ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alport</td>
<td>Reduced interstitial fibrosis</td>
<td>No improvement of renal function</td>
<td>[66]</td>
</tr>
<tr>
<td>5/6 nephrectomy</td>
<td>Reduced proteinuria</td>
<td></td>
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Overall, there is evidence for the presence of resident renal SC that may contribute to renal recovery after AKI. The SP functionally and phenotypically. They reported the isolation of a heterogeneous SP with an expression pattern similar to proximal tubular cells and the ability to integrate into embryonic kidneys, although not forming entire structures. More recently, the isolation of multipotent renal progenitor cells from adult rat kidneys has been described [47]. The cells expressed CD90, CD44, Pax2, Oct4, vimentin and could be passaged for >200 passages. When injected into the renal capsule of Fisher rats, multipotent renal progenitors integrated into tubular structures of normal and ischemic tissue. Gupta and co-workers proposed that these progenitors are kidney stem cells that localize to the renal tubule. In fact, Kitamura et al. had already reported a rat progenitor renal cell line, rKS56, derived from the S3 segment of proximal tubules [48]. rKS56 cells express both immature renal and mature tubular cell markers. In vitro, they were able to differentiate into tubular cells and to engraft to the kidney in a rat ischemic reperfusion model, were they replaced injured tubules and helped to improve renal function. Nonetheless, the presence of a nontubular multipotent Sc-1 positive stem population in a mouse kidney has also been published [49]. When injected in vivo after ischemia, some Sc-1+ cells were found to be scattered among the tubule. Improvement of renal function was not assessed.

In the human kidney, the first report of a resident progenitor population came from our group [50]. Via magnetic cell sorting, we isolated cells positive for antigen CD133 from adult human kidneys. These cells were negative for haematopoietic markers but expressed the MSC CD29, CD73 and the embryonic renal marker Pax2. CD133+ cells were clonogenic and were able to differentiate into epithelial and endothelial lineages when cultured under specific conditions. To clarify their role in renal regeneration, CD133+ cells were injected intravenously in the glycerol induced AKI model. After 3 days, CD133+ cells were detected in the proximal and distal tubules of injured kidneys, suggesting a role in renal repair. CD133+ cells have also been isolated from the Bowman’s capsule [51]. When injected into mice with glycerol induced AKI these cells were found to localize preferentially to tubule and improved renal function.

Overall, there is evidence for the presence of resident renal SC that may contribute to renal recovery after AKI. The
possibility to modulate proliferation/differentiation of the resident stem cells pharmacologically is of great interest. Recently, the therapeutic effect of a histone deacetylase inhibitor was shown to depend on the modulation of SP cells in a murine model of nephrotoxic serum-induced nephritis [52].

**GLOMERULAR INJURY**

Glomerulonephritis (GN), an injury of the renal glomeruli, is caused by immune and non-mediated diseases that ultimately lead to proteinuria and loss of functional nephrons. Injury to the podocytes, the final barrier in renal filtration, is a key element in nephrotic GN [53]. Podocyte damage and loss is frequently associated with a loss of microvasculature, which is strongly correlated with glomerular scarring [54]. Further characteristics of glomerular injury are reduced expression of angiogenic factors, increased synthesis of anti-angiogenic factors and a reduced proliferative response of the endothelium [54]. Vascular endothelial growth factor (VEGF) seems to reduce the progression of glomerular injury and might enhance glomerular repair [55, 56]. Remodelling of the glomerulus after injury therefore faces several problems, (i) regeneration of renal capillaries, (ii) remodelling of the extra cellular matrix produced during the scarring process and (iii) replacement of podocytes. Thus the regenerative process strongly depends on the regenerative potential of each cell type involved. The influence of SC on the recovery or progression of GN is not well studied. So far, most of the research focused on aspects of glomerular angiogenesis using the Thy-1 model of glomerulonephritis, a well described rat model of experimental mesangial proliferative glomerulonephritis. In this model, the injection of anti Thy-1 antibody causes complement activated lysis and injury of the glomerular mesangial cells, with consequent loss of the glomerular capillaries. A further characteristic are aneurismatic structures and alterations of the capillary architecture. The induced glomerular damage is subsequently repaired by proliferation and migration of remaining mesangial cells and by neo-angiogenesis [57].

**Endogenous BMSC in the Regeneration of Glomerular Injury**

Transplantation of BM into rats with Thy-1 GN has been shown to support the regeneration of renal capillaries [18]. The capacity of SC to regenerate the glomerulus from adverse extra cellular matrix production during GN was demonstrated in a mouse model for Alport syndrome. Injecting BM into irradiated collagen 4A3 knock out mice improved the reconstitution of the glomerular architecture with a partial restoration of the expression of the type IV collagen alpha3 chain [58]. Sugimoto et al. [58] found that BM derived epithelial cells were recruited to the glomerular basement membrane, suggesting a function as podocytes. Similar results were obtained by Prodromidi et al. [59] that also showed the differentiation into podocytes and mesangial cells of BM-derived progenitor cells. Rookmaaker et al. demonstrated the revascularization potential of BM-derived SC following Thy-1 injury [60]. They found that SC repopulate mesangial and endothelial cells of glomeruli [19, 60].

In all these studies of BM-derived SC recruitment to damaged glomeruli, the lineage of SC recruited has not been established.

**Administration of BMSC in the Regeneration of Glomerular Injury**

Several studies showed a beneficial effect of exogenous BMSC treatment on acute experimental glomerulonephritis using both EPC and MSC (Table 1). EPC derived from BM and injected in rats with experimental Thy-1 GN promoted the recovery from glomerular injury and increased revascularization [61]. In addition, EPC were found to integrate in the endothelial lining [61, 62]. Exogenous MSC also showed a positive effect on the recovery from experimental glomerulonephritis [60]. The injection of MSC induced a faster re-

<table>
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<tr>
<th>Title of Study</th>
<th>Pathology</th>
<th>Cell Type</th>
<th>Control – Phase of Trial</th>
<th>Time</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchymal Stem Cells and Subclinical Rejection</td>
<td>Kidney Transplantation</td>
<td>MSC</td>
<td>Uncontrolled Phase I + II</td>
<td>10/2008 - 12/2010</td>
<td>NCT00734396</td>
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*ClinicalTrials.gov Identifier.
covery from Thy-1 GN. MSC injected in rats with Thy-1 GN also exhibited an increased production of VEGF and TGF-ß [63, 64].

In contrast, long-term experiments of SC injection showed some side or negative effects. Wong et al. injected human MSC in a murine model for experimental GN induced by an anti-mesangial antibody. Injected exogenous MSC differentiated into mesangial cells in the glomerulus [65]. While the injected MSC had an immediate positive effect, they negatively contributed to the long-term regeneration of the glomerular tissue and induced progression into glomerulosclerosis. In another study performed by Kunter et al. the injected MSC in a long-term analysis of the renal tissue were found to undergo maldifferentiation in adipocytes [63].

Regarding chronic renal failure, Ninichuk et al. [66] reported that MSC administration reduced interstitial fibrosis, but did not delay the progression of chronic kidney disease in the model of Alport syndrome. Prodromidi et al. [59] also showed that a single MSC administration did not improve renal function in the murine model of Alport syndrome.

In a 5/6 nephrectomy model of chronic renal failure, MSC administration did not improve renal function, but it reduced proteinuria [67].

CONCLUSION

In conclusion, preclinical studies suggest that the administration of exogenous SC may ameliorate AKI and accelerate regeneration (Table 1). In consideration of the role of endogenous BMSC, a possible approach could also be the SC mobilization. However, the possible effects of BM-recruited cells and of inflammatory cells in this experimental setting require further investigation. Currently, the most promising approach is considered to be the administration of in vitro expanded MSC applied to acute tubular and glomerular injury. Injected MSC were shown to home to the injured kidney and to accelerate morphological and functional regeneration, possibly by a paracrine or even endocrine mechanisms, although their engraftment and transdifferentiation was not observed in the majority of the studies. A major role in the effect of MSC has been attributed to the production of growth factors and cytokines with immunosuppressive, anti-inflammatory, anti-apoptotic and proliferative effects. Several clinical trials have been designed or are in progress to evaluate the effect of MSC administration in renal transplantation, acute renal injury or chronic allograft nephropathy (www.clinicaltrial.gov) (Table 2). The effect of MSC administration in chronic renal damage still deserves investigation.

In addition, different populations of resident SC have been identified in the adult kidney. It is not clear whether these cells are involved in the physiological cell turn-over or in the regeneration of renal injury. The possibility to expand and differentiate local progenitors or SC is another interesting approach for regenerative medicine. However, it is still necessary to increase our understanding of SC mechanisms and, in the view of possible maldifferentiation or transformation, to evaluate the safety of their use in long-term therapy, such as in chronic renal failure.

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